SUPPORTING INFORMATION

for

Melting of a β-hairpin peptide using isotope-edited 2D IR spectroscopy and simulations

*Adam W. Smith,[†] Joshua Lessing, Ziad Ganim, Chunte Sam Peng, and Andrei Tokmakoff**

Department of Chemistry, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA 02139 USA

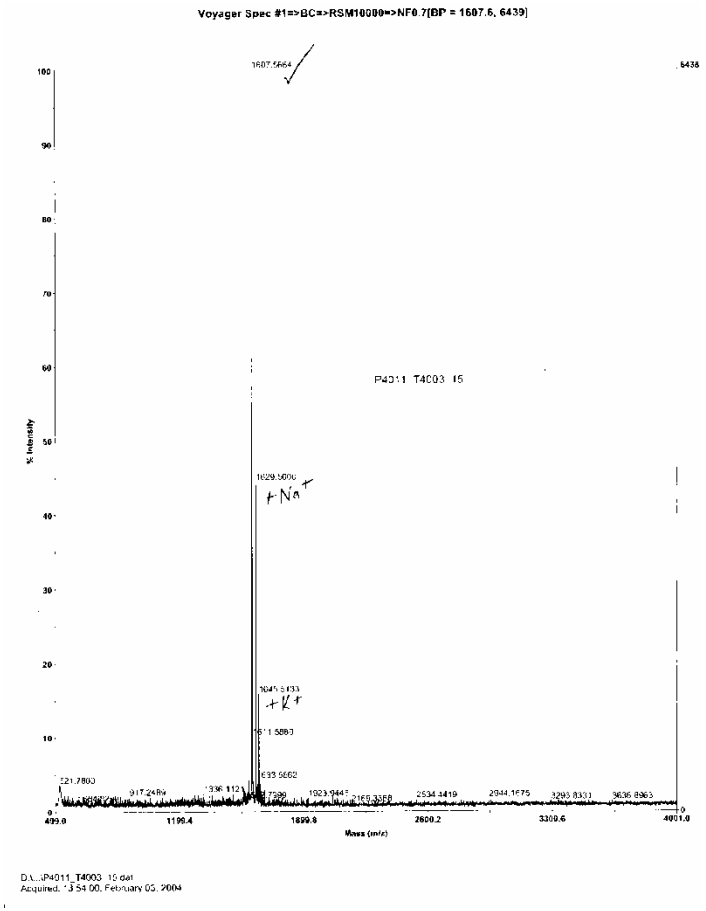
Santanu Roy, Thomas L. C. Jansen, and Jasper Knoester

Center for Theoretical Physics and Zernike Institute for Advanced Materials, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands

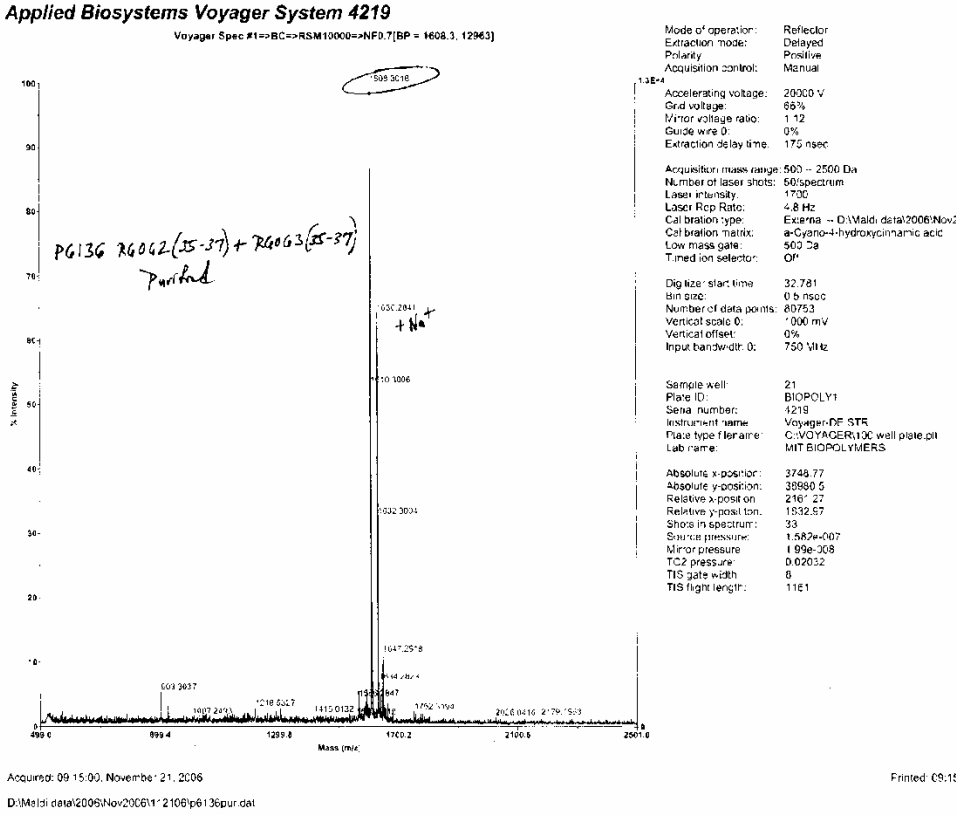
Contents:

1. Mass spectra of the synthesized peptides

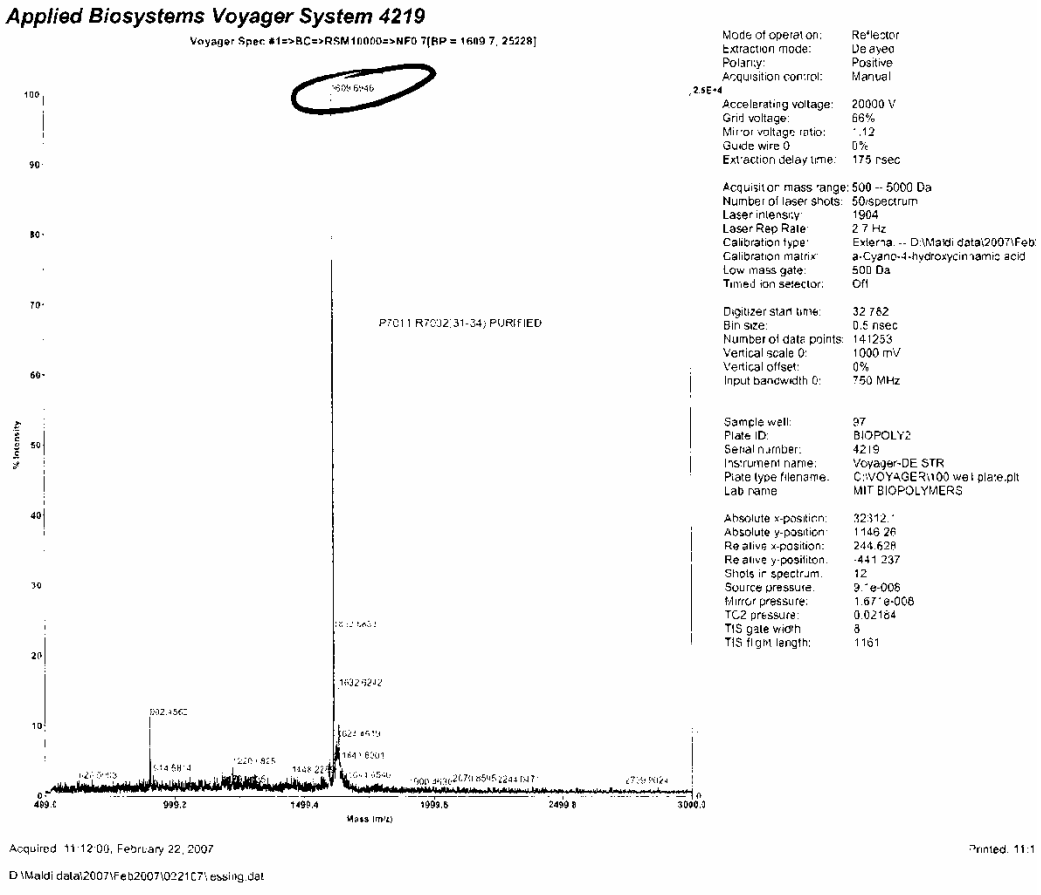
1.a. TZ2-UL mass spectrum recorded on an Applied Biosystems Voyager MALDI-TOF mass spectrometer on February 3, 2004. The expected mass of TZ2-UL is 1608 g/mol, and the recorded mass is 1607.6 g/mol.



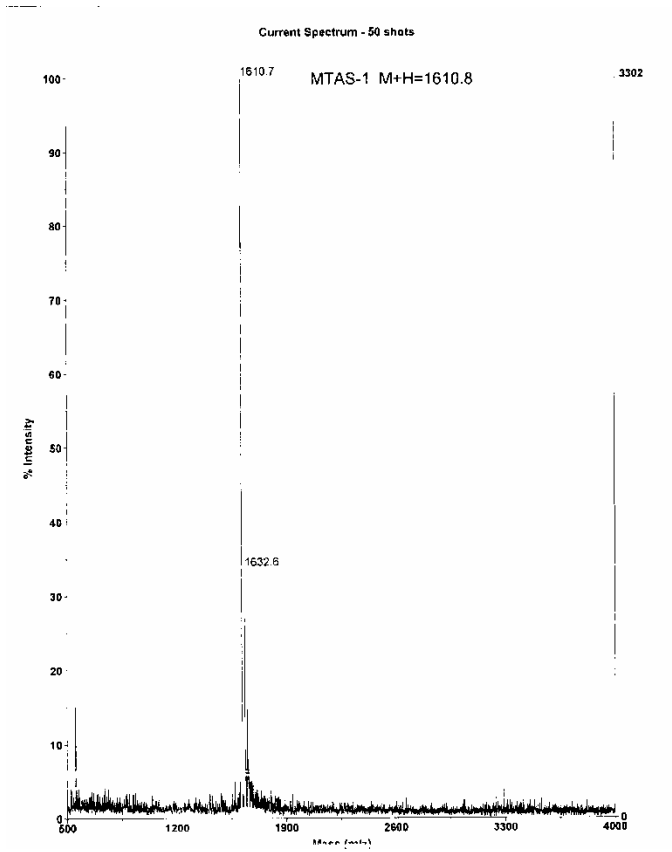
1.b. TZ2-K8 mass spectrum recorded on an Applied Biosystems Voyager MALDI-TOF mass spectrometer on November 21, 2006. The expected mass of TZ2-K8(^{13}C) is 1609 g/mol. The recorded mass is 1608.3 g/mol, which is approximately 1 a.m.u. higher than the recorded mass of TZ2-UL (Fig. 2.a.1).



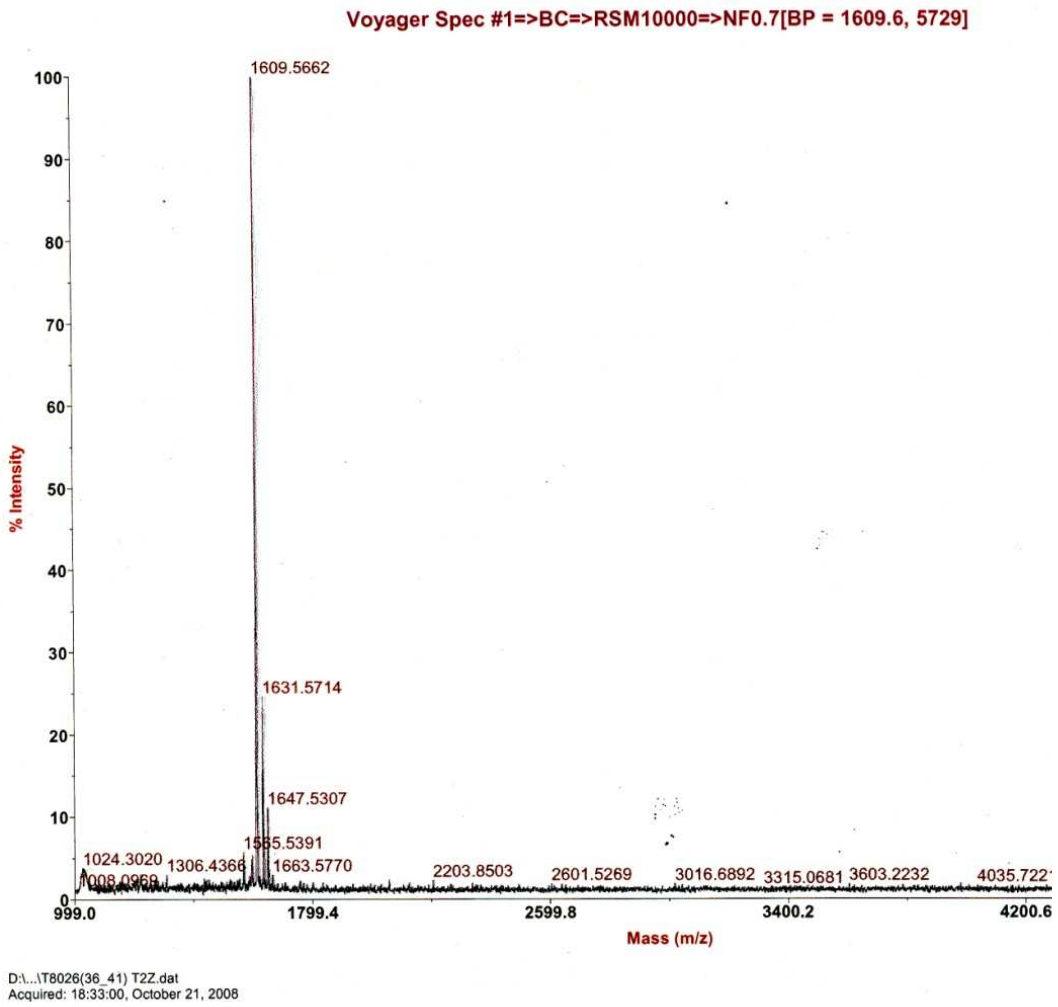
1.c. TZ2-S1 mass spectrum recorded on an Applied Biosystems Voyager MALDI-TOF mass spectrometer. The expected mass of TZ2-S1(^{18}O) is 1610 g/mol. The recorded mass is 1609.6 g/mol, which is approximately 2 a.m.u. higher than the recorded mass of TZ2-UL.

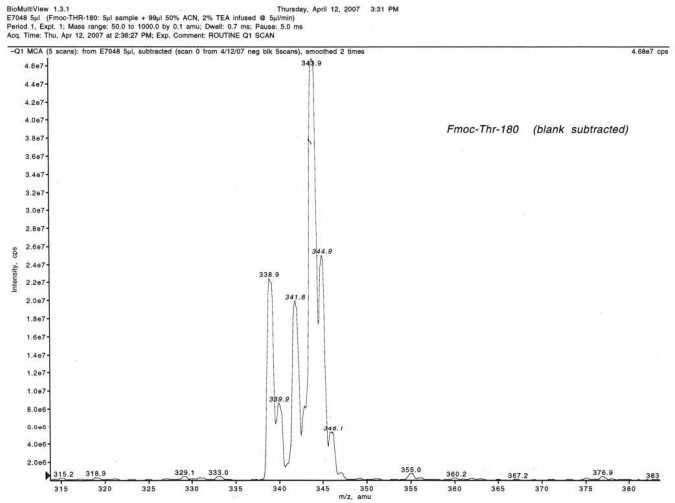


1.d. TZ2-TT mass spectrum provided by Anaspec Inc. The expected mass of TZ2-T3(^{13}C)T10(^{13}C) is 1610 g/mol, which agrees with the recorded mass of 1610.7.

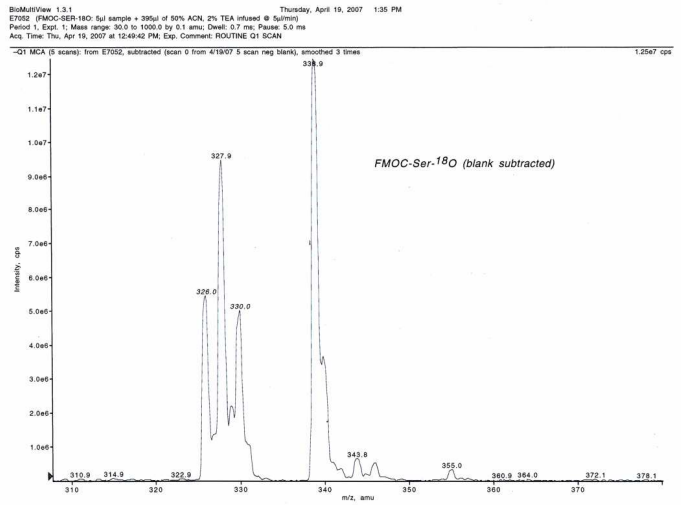


1.e. TZ2-T10 mass spectrum recorded on an Applied Biosystems Voyager MALDI-TOF mass spectrometer. The expected mass of TZ2-T10(^{18}O) is 1610 g/mol. The recorded mass is 1609.6 g/mol, which is approximately 2 a.m.u. higher than the recorded mass of TZ2-UL.





1.f. 18O labeled Fmoc-Thr-OH mass spectrum recorded on a Bruker Daltonics APEXIV 4.7 Tesla Fourier Transform Ion Cyclotron Resonance Mass Spectrometer (FT-ICR-MS). The expected mass of unlabeled Fmoc-Thr-OH is 341.4 g/mol. The recorded masses are 341.8, 343.9, 344.9, and 346.1 g/mol. These masses represent a distribution in isotople label incorperation into Fmoc-Thr-OH.

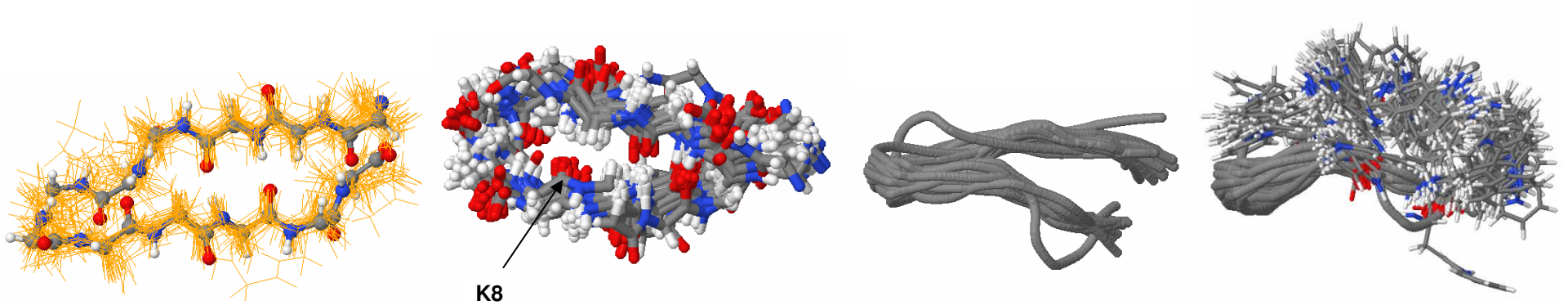


1.g. 18O labeled Fmoc-Ser-OH mass spectrum recorded on a Bruker Daltonics APEXIV 4.7 Tesla Fourier Transform Ion Cyclotron Resonance Mass Spectrometer (FT-ICR-MS). The expected mass of unlabeled Fmoc-Ser-OH is 327.3 g/mol. The recorded masses are 326.0, 327.9, and 330.0 g/mol. These masses represent a distribution in isotople label incorperation into Fmoc-Ser-OH.

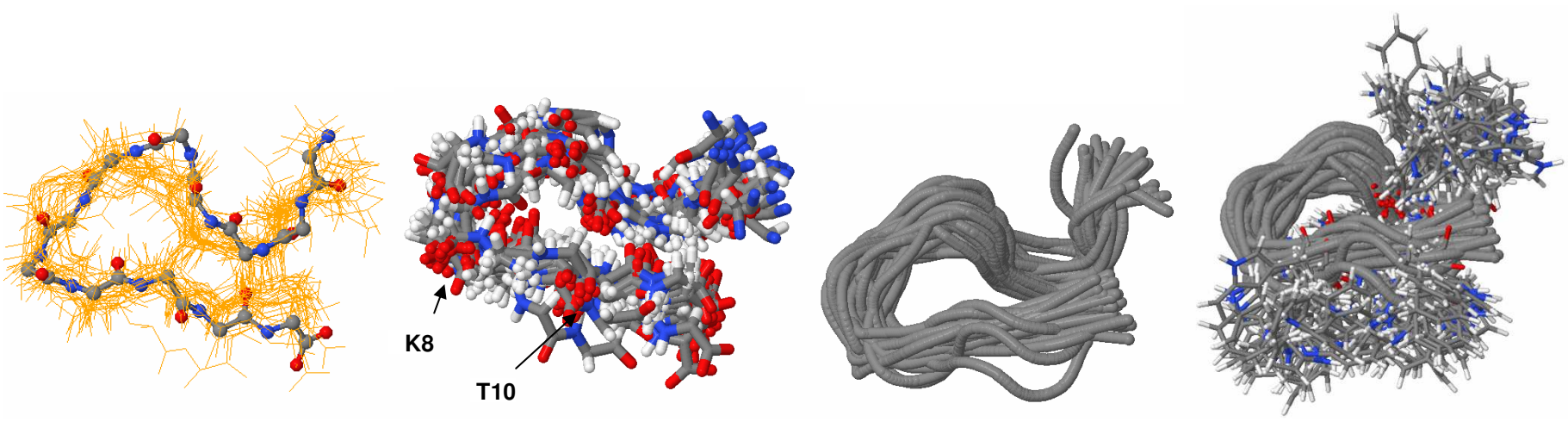
2. Visualization of the Markov States: Backbone conformation and tryptophan packing

Here we provide different visualizations of TZ2 for the 30 initial structures within each simulated Markov state. Backbone visualizations serve to characterize the amide group orientations and conformational disorder of each state, and the right panel illustrates the packing of tryptophans for each state. Structures are oriented in a manner that coincides roughly with Fig. 9.

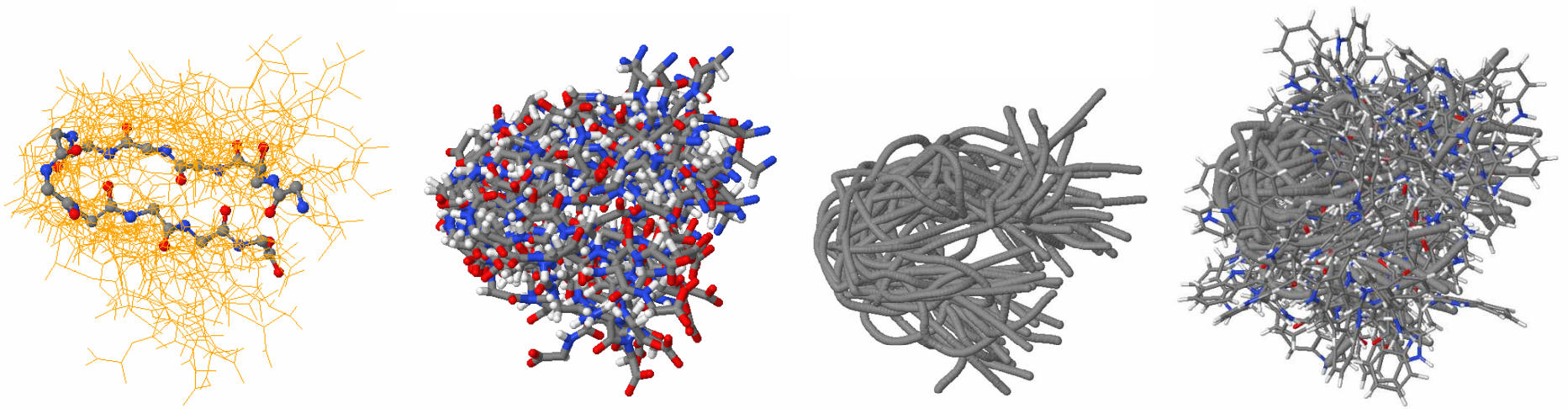
"Folded" (**FO,** 250851)



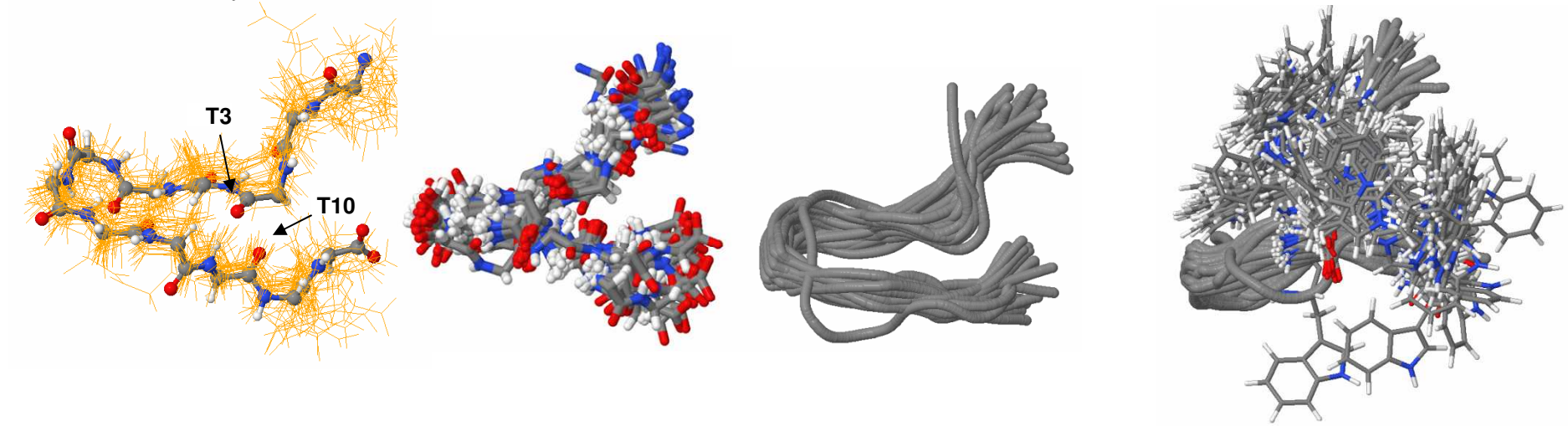
"Bulged" (**BT,** 214369)



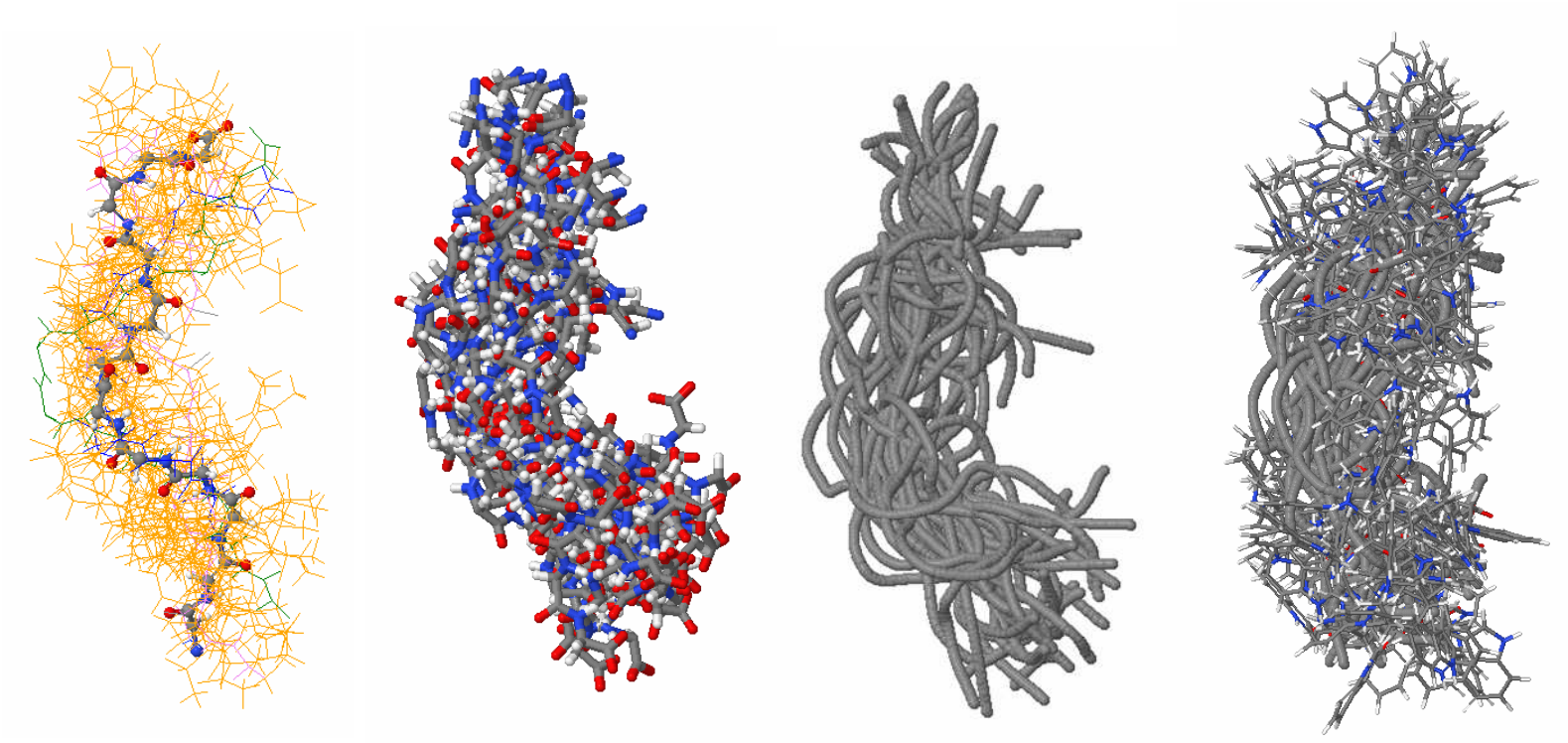
"Disordered" (**SR**, 271154)



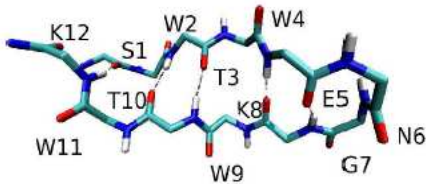
"Frayed" (**FR,** 11131)

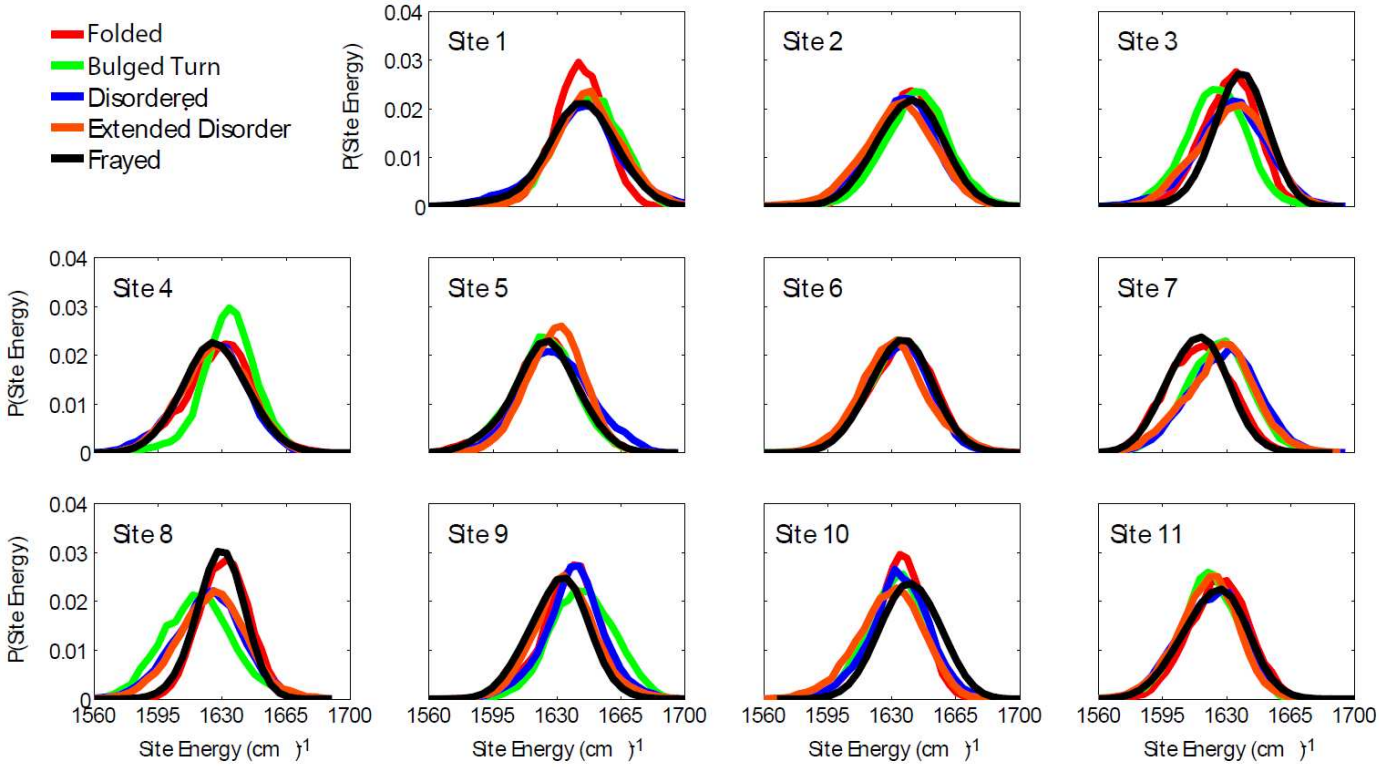


“Extended Disorder” (**ED,** 64336)

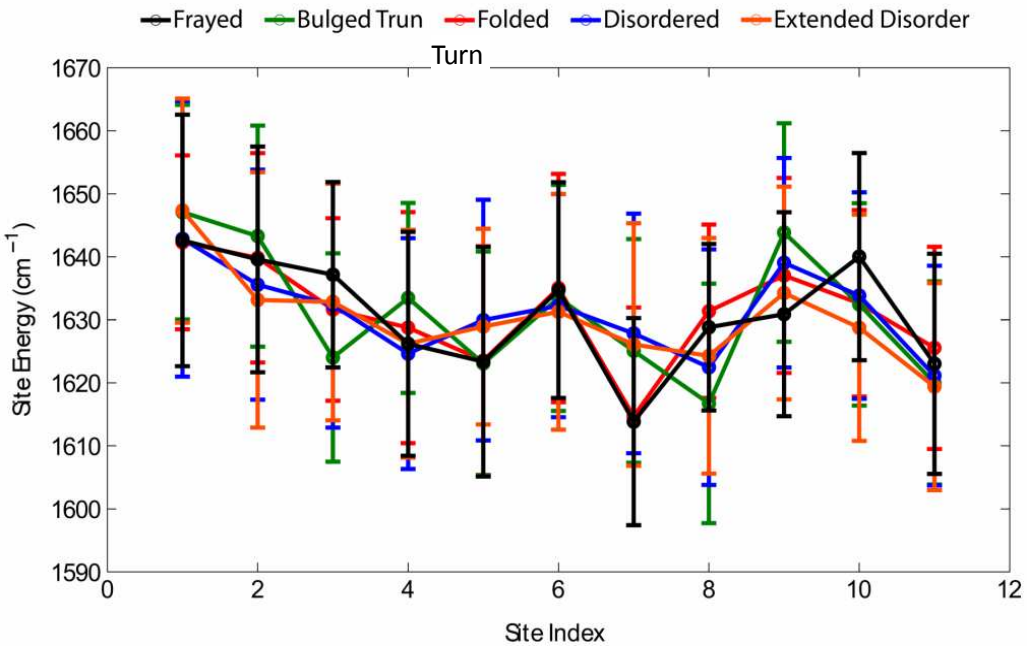


3. Analysis of simulated site energy variation by conformer

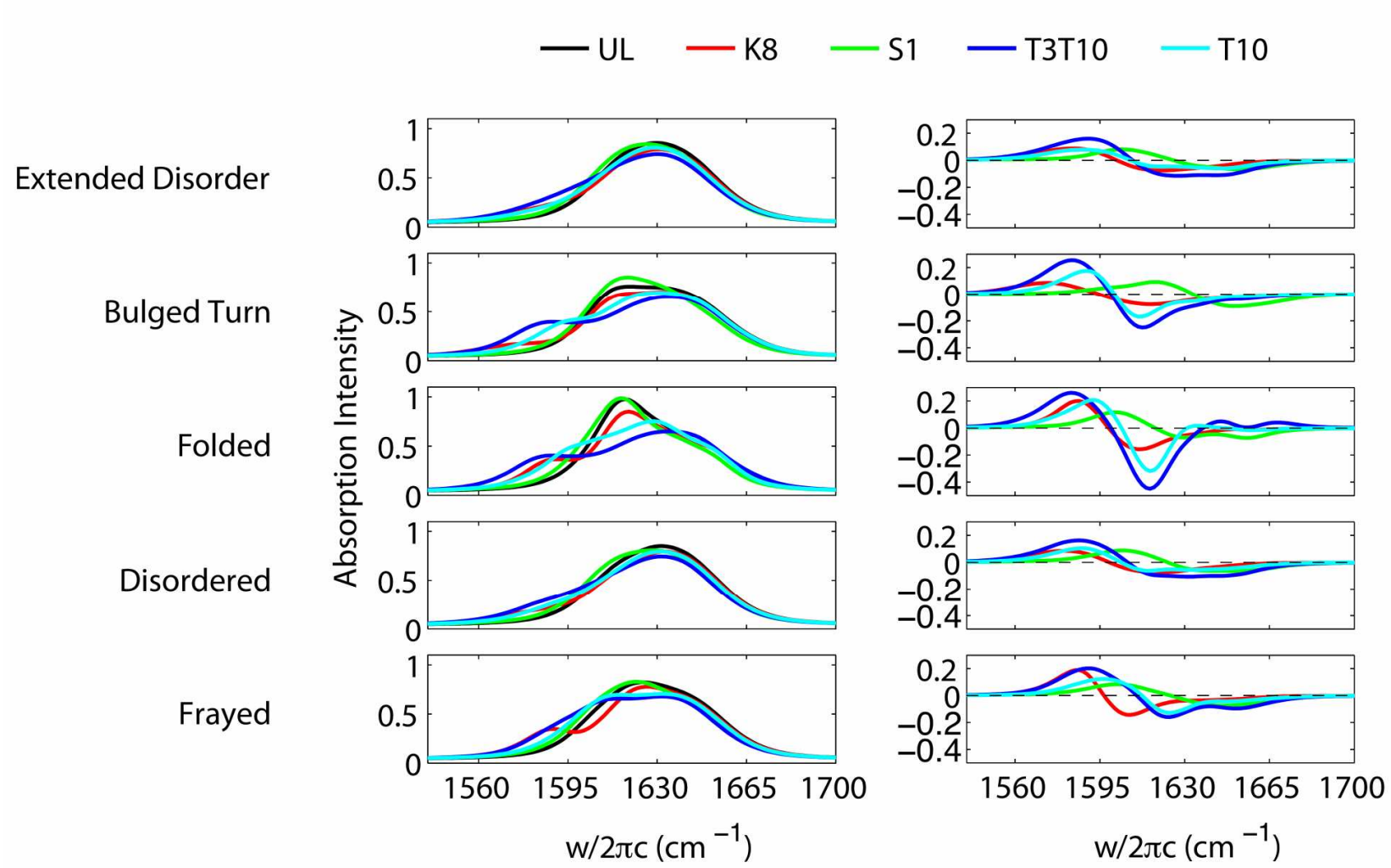




Site energy mean values showing error bars with one standard deviation



4. Simulated FTIR and FTIR difference spectra for isotopologues of all Markov States



5. Comparison of spectral features in simulations with experiment.

All units in cm^{-1}.

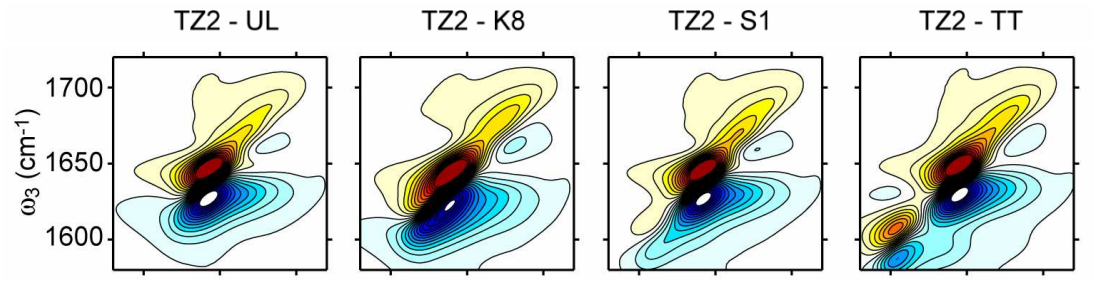
	Experiment (25°C)					Experiment (75°C)					Simulations											
											64336	Frayed (FR)		214369	Bulged (BT)		250851	Folded (FO)		271154	Disordered (SR)	
	Absorbance Maximum			FWHM^		Absorbance Maximum			FWHM		Peak		Width	Peak		Width	Peak		Width	Peak		Width
Site	Ex*	Iso**	Iso2	Iso	Iso2	Ex*	Iso**	Iso2	Iso	Iso2	Ex	Iso	Iso	Ex	Iso	Iso	Ex	Iso	Iso	Ex	Iso	Iso
UL	1636					1637					1649			1645			1651			1646		
S1	1633	1629		10		1635					1647	1637	4.6	1660	1647	6.5	1651	1635	5.7	1646	1639	6.8
K8	1635	1598	1615	22	10	1639	1601	1617	17	10	1652	1623	13.7	1644	1608	8.3	1651	1622	5.7	1645	1616	10.8
T 10	1639	1628		9		1644	1632		9		1649	1624	9.8	1653	1628	6.3	1640	1627	3.3	1649	1624	8.3
TT	1635	1603		19		1634	1602		17		1651	1629	13.1	1629	1616	6.6	1645	1622	6.0	1647	1622	10.9

* Excitonic ^{12}C band absorbance maximum

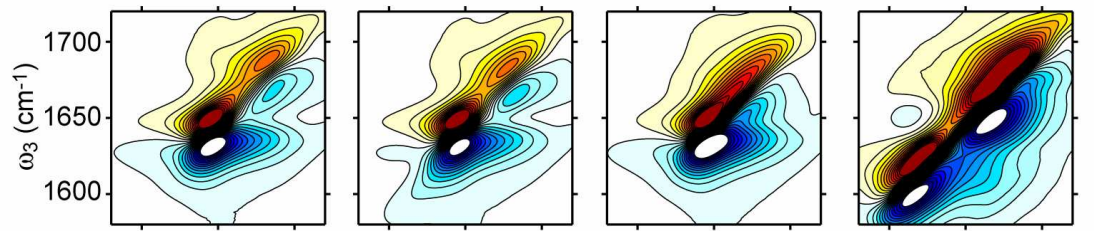
** Isotope shifted peak absorbance maximum

^ Diagonal peak width (FWHM)

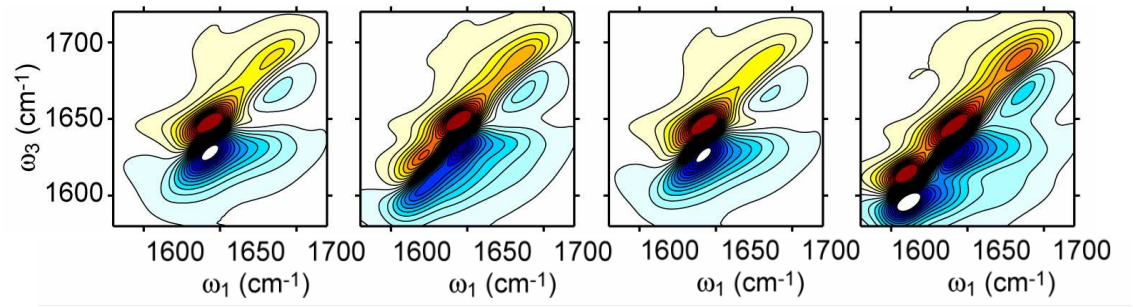
6. 2D IR spectral simulations of TZ2 isotopologues for specific conformations



NL simulation for single structure with intact S1-W12 hydrogen bond.



Slipped registry simulation for a single +1C slipped registry conformer with 4 hydrogen bonds.



Simulations of the 2D IR spectra for the NMR structure of TZ2.